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A Short Total Synthesis of *rac*-Peniolactol

Aamer Saeed*

Department of Chemistry, Quaid-I-Azam University, Islamabad-45320, Pakistan

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Summary. A short facile synthesis of the fungal metabolite (±)-3,4-dihydro-3,6,8-trihydroxy-3-pentadecylisocoumarin (peniolactol) has been achieved. Condensation of hexadecanoyl chloride with 3,5-dimethoxyhomophthalic acid afforded 6,8-dimethoxy-3-pentadecylisocoumarin, which on sequential saponification and demethylation furnished *rac*-peniolactol in 31% overall yield. The ring-chain tautomerism was studied in solution by ^{1}H NMR and the mass fragmentation pattern. 3-Pentadecylisocoumarin was also synthesized and saponified to the corresponding keto acid as a model compound for comparative studies.

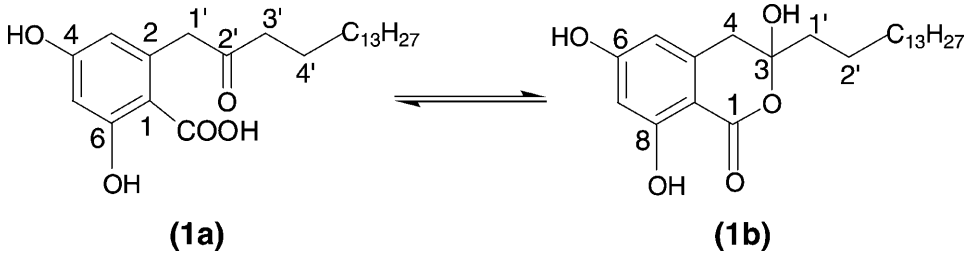
Keywords. *rac*-Peniolactol; *Peniophora sanguinea*; Isocoumarins; Ring-chain tautomerism.

Introduction

Peniolactol [1] was isolated from the wood attacked by the fungus *Peniophora sanguinea* Bres. and was assigned the constitution of 3,4-dihydro-3,6,8-trihydroxy-3-pentadecylisocoumarin. It can exist in two tautomeric forms: the open chain keto acid (**1a**) and the cyclic hemiacetalic lactol form (**1b**) (Scheme 1). This feature is also exhibited by other 3-hydroxy-3-alkyl-3,4-dihydroisocoumarins like ustic acid [2], sclerotinin A and B [3], 3-hydroxymellein, 3,4,6-trihydroxymellein [4], 3,6-dihydroxymellein [5], and the lactols of the biogenetic precursors of cochlioquinones and stemphone [6]. Presumably, owing to this tautomeric interconversion at the prochiral center peniolactol occurs as a racemate unlike other 3-substituted dihydroisocoumarins, the majority of which occur in nature as the (*R*)-enantiomers [7–9].

We have already reported on the total synthesis [10] of *rac*-peniolactol by a multistep strategy developed during the synthesis of the main dihydroisocoumarins [11] of the plant *Ononis natrix*. Peniolactol is a potential antifungal and antibacterial agent, thus a detailed study of the biological activity required a short and efficient synthetic route. Condensation of acid chlorides with homophthalic acids has recently emerged as a standard and authentic route for the construction of the

* E-mail: aamersaeed@yahoo.com



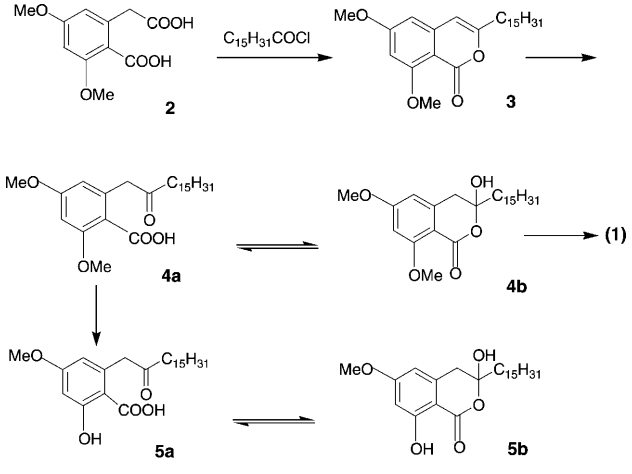
Scheme 1

3-substituted isocoumarin skeleton. Herein, a facile three step synthesis of peniolactol using this method will be described.

Results and Discussion

The key starting substance for the synthesis of *rac*-peniolactol, 3,5-dimethoxyhomophthalic acid (**2**) was prepared from the corresponding phenylacetic acid according to the procedure reported by us earlier [12]. Direct reaction of 3,5-dimethoxyhomophthalic acid with hexadecanoyl chloride at elevated temperature afforded 6,8-dimethoxy-3-pentadecylisocoumarin (**3**) in 80% yield [13] (Scheme 2). This compound showed the characteristic 1H singlet of the isocoumarin moiety at $\delta = 6.06$ for H-4 in the ^{1}H NMR and signals at $\delta = 99.71$(C-4) and 159.54 (C-3) in the ^{13}C NMR spectrum. The mass spectrum showed the characteristic isocoumarin fragment ion at m/z 177, and in its IR spectrum the lactonic carbonyl absorption was observed at 1734 cm^{-1}. 3-Pentadecylisocoumarin (**3a**), prepared similarly from commercial homophthalic acid gave a singlet at $\delta = 6.24$ for H-4 and signals at $\delta = 103.91$ (C-4) and 158.51 (C-3) in the ^{1}H and ^{13}C NMR spectra. The IR spectrum showed the lactonic carbonyl absorption at 1728 cm^{-1}.

Alkaline hydrolysis of the isocoumarin **3** to furnish 4,6-dimethoxy-2-(2-oxoheptadecyl) benzoic acid (**4a**) was accomplished in good yield. The keto acid



Scheme 2

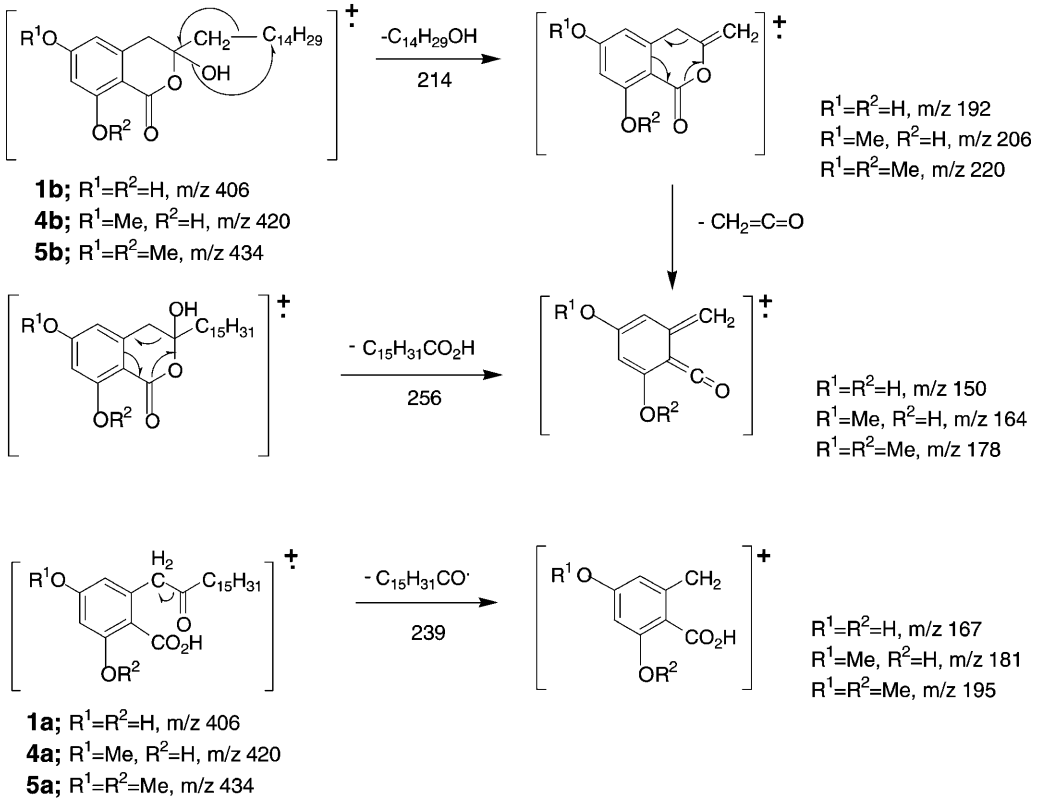
existed partially in equilibrium with its cyclic tautomeric form 6,8-dimethoxy-3-hydroxy-3-pentadecyl-3,4-dihydroisocoumarin (**4b**) as evidenced by the diastereotopy of prochiral benzylic protons in the ^{1}H NMR spectrum. Thus, in addition to a 2H singlet at $\delta = 4.05$ (H-1′ open chain form **4a**), each of the protons of $ArCH_2$ showed a double doublet AB pattern at $\delta = 3.60$–3.80, $J = 7.1$ Hz (H-4 lactol form **4b**) in the ^{1}H NMR and at $\delta = 50.23$ (C-1′ open chain) in the ^{13}C NMR spectrum. DEPT 90° and DEPT 135° experiments confirmed these assignments. The mass spectrum showed the characteristic M^+-H_2O peak at m/z 416. The IR spectrum showed the carboxyl and ketonic carbonyl absorptions at 1683 and 1716 cm^{-1}, respectively. The unsubstituted keto acid **4c** obtained from the hydrolysis of 3-pentadecylisocoumarin showed a sharp 2H singlet at $\delta = 4.10$ or a mutiplet at $\delta = 4.01$–4.18 and 77.29 (C1′) in the ^{1}H and ^{13}C NMR spectra.

Selective demethylation of the 4,6-dimethoxy keto acid lactol tautomeric mixture **4ab** using BBr_3 occurred rapidly under mild conditions (-78°C, 10 min) to furnish 6-hydroxy-4-methoxy-2-(2-oxoheptadecyl) benzoic acid (**5a**), which predominantly existed as 3,8-dihydroxy-6-methoxy-3-pentadecyl-3,4-dihydroisocoumarin (**5b**) as shown by a quartet at $\delta = 2.91$–3.25, $J = 8.54$ Hz as compared to a very feeble singlet at $\delta = 3.95$ for the keto acid form.

The crucial step was the final deprotection to obtain **1**. Although BBr_3 is the reagent of choice for complete cleavage of *O*-methyl ethers of natural products, in case of a keto acid like **4a**, which is highly acid sensitive, the use of a strong *Lewis* acid may lead to side reactions, the major competing side reaction being the acid catalyzed dehydration to the parent dimethoxyisocoumarin **3**. If the dehydration would occur after complete or partial demethylation instead of peniolactol the corresponding isocoumarins would have resulted. Fortunately, the only major side product was the parent dimethoxy isocoumarin **3**, which could easily be separated and recycled.

The *rac*-peniolactol was characterized by the complete absence of *Me*O-8 and *Me*O-6 singlets, both in the ^{1}H NMR and ^{13}C NMR spectra, and by the downfield shift of the characteristic $ArCH_2$ singlet from 4.05 to 3.94 ppm (open chain **1a**) or the quartet at 2.86–2.65 ppm, $J = 7.52$ Hz (lactol form **1b**) in addition to other characteristic changes. Thus, the present synthesis constitutes a highly simplified route to *rac*-peniolactol (**1**) involving three linear steps. It proceeds with a 31% overall yield, which makes **1** available for biological evaluation. Peniolactol is expected to possess potent antifungal and antibacterial activities as shown by the related isocoumarins [14]. These studies are in progress.

Some interesting observations regarding the interconversion of keto acid and lactol forms could be made. Previous studies on such keto acid lactol equilibria have reported that keto acids with a methoxy group adjacent to the carboxyl exist mainly in the open chain form, whereas those with a hydroxy substituent at this position predominantly exist in the lactol form. This is possibly due to internal chelation, both in solution and solid state as shown by their ^{1}H NMR and X-ray structures [5, 15]. In the present study it was observed that initially the dimethoxy keto acid **4a** existed only in the keto acid form (2H singlet at $\delta = 4.05$ for CH_2-1′) but on standing appreciable conversion to the lactol form **4b** occurred as shown by the clean eight line AB pattern (dddd) of the *Ar*-CH_2 protons at $\delta = 3.60$–3.80, $J = 7.1$, 6.1 Hz most probably due to ^4J coupling with the aromatic proton. Partial



Scheme 3

demethylation to the hydroxy keto acid **5** also gave an AB pattern at $\delta = 3.25–2.91$, $J = 8.54$ Hz but only four lines were visible showing that it mainly existed in the lactol form **5b**. In case of peniolactol again initially a singlet at $\delta = 3.94$ (open chain **1a**) was observed which became a quartet at $\delta = 2.86–2.65$, $J = 7.4$ Hz (**1b**). These observations indicated that although in the solid state the 2-hydroxy keto acids may exist exclusively in the lactol form, in solution ($CDCl_3$) interconversion between both forms continues and the dimethoxy acids may also exist as tautomeric mixture of two forms in accordance with a recent report on similar keto acids [16]. Even for the unsubstituted keto acid **4c** initially a sharp singlet at $\delta = 4.10$ was observed which became a mutiplet at $\delta = 4.01–4.18$ on standing indicating that these are also prone to tautomerization. The mass fragmentation pattern of **1**, **4**, and **5** also support their existence in the lactol form. Thus, in each case the relative intensity of cation radicals obtained from the lactol form by elimination of $C_{14}H_{29}OH$ and $C_{15}H_{31}CO_2H$ was much higher as compared to that obtained from the open chain keto acid form due to elimination of the pentadecanoyl group (Scheme 3).

Experimental

Commercial ethyl acetate and petroleum ether (60–80°C) were distilled before use, CH_2Cl_2 was dried over CaH_2 and distilled. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker AM-400 and AM-100 instruments, respectively, and J values are reported in Hz. IR spectra were recorded on a

Bruker Vector 22, the mass Spectra (EI, 70 eV) on a MAT 312 instrument, and the elemental analyses with a CHN-Rapid Heräus. The obtained values agreed favourably with the calculated ones. Flash Column chromatography (FCC) was carried out on Merck Kieselgel 60 (230–400 mesh).

6,8-Dimethoxy-3-pentadecylisocoumarin (**3**, $C_{26}H_{40}O_4$)

A stirred mixture of 0.5 g **2** (2.08 mmol) and 2.29 g hexadecanoyl chloride (8.33 mmol) was heated on an oil bath at 200°C for 3 h. FCC of the residue (petroleum ether:ethyl acetate = 8:2) afforded a white solid which on recrystallization from methanol gave 0.69 g isocoumarin **3** (80%) as colourless prisms. Mp 101–103°C (Ref. [10] 102 –103°C); MS (70eV): m/z (%) = 416 (M^+); IR (film): $\nu = 2913$, 2849, 1734, 1694, 1598, 1572, 1471, 1151, 832 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.86$ (t, $J = 7.12$ Hz, 3H, H-15′), 1.23 (br s, 24H, H3′–H14′), 1.67 (p, $J = 8.4$ Hz, 2H, H2′), 2.44 (t, $J = 7.0$ Hz, 2H, H-1′), 3.87 (s, MeO-8), 3.93 (s, MeO-6), 6.06 (s, H-4), 6.30 (d, $J = 2.12$ Hz, H-5), 6.40 (d, $J = 2.12$ Hz, H-7) ppm; ^{13}C NMR ($CDCl_3$,100 MHz): $\delta = 165.8$ (C1), 163.6 (C8), 159.5 (C6), 142.9 (4a), 103.1 (C4), 98.4 (C7), 99.7 (C5), 56.5 (MeO-8), 55.8 (MeO-6), 33.6 (C1′), 32.1 (C13′), 29.88, 29.86, 29.77, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1 (C4′–C12′), 27.0 (C2′), 23.7 (C3′), 22.9 (C14′), 14.3 (C15′) ppm.

3-Pentadecylisocoumarin (**3a**, $C_{24}H_{36}O_2$)

Prepared in a manner similar to **3** from commercial homophthalic acid and the product flash chromatographed from pure pet ether to afford **3a** as colourless needles in 82% yield. Mp 58–59°C; MS (70 eV): m/z (%) = 356 [M^+] (97.7), 336 (34.2), 314 (63.7), 160 (100), 118 (97.7); IR (film): $\nu = 2918$, 2849, 1728, 1712, 1656, 1604, 1160, 642 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.87$ (t, $J = 6.28$ Hz, 3H, H-15′), 1.28 (br s, 24H, H3′–H14′), 1.70 (p, $J = 8.4$ Hz, 2H, H2′), 2.52 (t, $J = 7.08$ Hz, 2H, H-1′), 6.24 (s, H-4), 7.34 (d, $J = 8.16$ Hz, H-5), 7.49 (td, $J = 0.88$, 7.28 Hz, H-7), 7.65 (m, H-6), 8.25 (d, $J = 8.16$ Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta =$ 163.2 (C1), 158.5 (C3), 137.8 (C8a), 134.8 (C6), 129.6 (C8), 127.6 (C7), 125.1 (C5), 120.3 (C4a), 103.9 (C4), 33.7 (C1′), 32.0 (C13′), 29.88, 29.86, 29.82, 29.80, 29.7, 29.70, 29.6, 29.5 (C3′–C12′), 27.0 (C2′), 22.8 (C14′), 14.2 (C15′) ppm.

2,4-Dimethoxy-6-(2-oxo-heptadecyl)-benzoic acid (**4a**) *or 6,8-Dimethoxy-3-hydroxy-3-pentadecyl-3,4-dihydroisocoumarin* (**4b**) ($C_{26}H_{42}O_5$)

A stirred solution of 0.5 g **3** (1.20 mmol) in ethanol 20 cm^3 was treated with 40 cm^3 5% KOH and the mixture refluxed for 4 h. After cooling the reaction mixture, most of the ethanol was rotary evaporated. Cold water (20 cm^3) was added and the mixture acidified with dil. HCl and extracted with 2×30 cm^3 dichloromethane. The organic phase was dried ($MgSO_4$), and the solvent evaporated under vacuum to leave **4ab** as a white solid. Recrystallized from *Me*OH as colourless scales, 0.36 g (70%). Mp 109–111°C (Ref. [10] 112–115°C); MS: m/z (%) = 434 [M^+] (24), 416 (42), 417 (37), 391 (11), 220 (13), 197 (17), 178 (100); IR (film): $\nu = 2915$, 2849, 1713, 1694, 1601, 1202, 1162 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.87$ (t, $J = 6.88$ Hz, 3H, H-17′), 1.25 (m, 24H, H3′–H14′), 1.54–1.67 (p, 2H, H4′), 2.58–2.62 (t, $J = 6.67$, 2H, H-3′), 3.60–3.80 (dddd, 2H, $J = 7.16$, 6.9 Hz H4 lactol form **4b**), 3.85 (s, *Me*O-4) 4.0 (s, *Me*O-6), 4.05 (s, 2H, H1′ keto acid form **4a**), 6.39 (s, H-3), 6.48 (s, H-5) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 207.83$ (C = O), 163.07 (COOH), 107.20 (C3), 160.36 (C4), 160.36 (C6), 111.78 (C1), 98.14 (C5), 55.70 (*Me*O-6), 57.03 (*Me*O-8), 50.21 (C1′), 42.98 (C3′), 32.06 (C15′), 29.82, 29.80, 29.77, 29.63 (C5′–C14′), 22.82 (C16′), 14.25 (C17′) ppm (C/H numbering according to keto acid form **4a**).

2-(2-Oxoheptadecyl) benzoic acid (**4c**, $C_{24}H_{38}O_3$)

Prepared in a manner similar to **4** from 3-pentadecylisocoumarin in 75% yield as colorless needles. Mp 79–80°C. MS: m/z (%) = 374 [M^+] (11), 356 (37), 221 (19), 160 (79), 135 (24), 118 (31); IR (film): $\nu = 2915$, 2849, 1713, 1694, 1601, 1202, 1162 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.87$ (t, $J =$ 6.64 Hz, 3H, H-17′), 1.27 (m, 24H, H3′–H16′), 1.67 (m 2H, H4′), 2.51 (brs, 2H, H-1′), 4.1 (s,

2H, H1′ keto acid) or 4.05–4.18 (p, $J = 7.16$ Hz, 2H, H-4 lactol), 7.20 (d, $J = 5.76$ Hz, H-3), 7.39 (td, $J = 0.88$, 8.04 Hz, H-4), 7.54 (dt, $J = 7.16$ Hz, H-5), 8.2 (d, $J = 8.04$ Hz, H-6) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 203.1$ (C2′), 197.9 (COOH), 132.7 (C3), 131.8 (C4), 127.4 (C5), 77.2 (C1′), 125.1 (C6), 33.7 (C15′) 32.0 (C3′), 29.84, 29.80, 29.77, 29.72, 29.5, 29.4, 29.3, 29.2, 29.1 (C5′–C14′), 24.6 (C5′), 22.8 (C16′), 14.2 (C17′) ppm (C/H numbering according to keto acid form **4a**).

6-Hydroxy-4-methoxy-2-(2-oxoheptadecyl) benzoic acid (**5a**) *or 3,4-Dihydro-3,8-dihydroxy-6-methoxy-3-pentadecylisocoumarin* (**5b**) ($C_{25}H_{40}O_5$)

A 1 *M* solution of BBr_3 in 1 cm^3 CH_2Cl_2 (1.1 mmol) was added dropwise to a stirred solution of 0.16 g **4ab** (0.32 mmol) at -78°C in 4 cm^3 dry CH_2Cl_2 under Ar. After stirring for 10 min the reaction mixture was poured into 50 cm^3 ice-water. The layers separated and the aqueous layer extracted with 2×30 cm^3 CH_2Cl_2 and then 30 cm^3 *Et*OAc. The combined organic phases were dried ($MgSO_4$) and concentrated. Flash chromatography (petrol ether:ethyl acetate = 7:3) afforded 0.1 g **5ab** as colourless prisms (75%) and some recovered isocoumarin **3**. Mp 119–120°C; MS: m/z (%) = 421 [M^+, 1] (11.3), 420 [M^+] (24), 402 (22), 377 (50), 240 (32), 164 (100), 137 (22); IR (film): $\nu = 3410$, 1716, 1631, 1583, 1157 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.87$ (t, $J = 6.40$ Hz, 3H, H-15′), 1.20 (brs, 24H, H3′–H14′), 1.54–1.67 (m, 2H, H2′), 1.85–1.97 (t, $J = 8.0$ Hz, 2H, H-1′), 2.91–3.25 (q, 2H, H-4, $J = 8.54$ Hz, lactol form **5b**), 3.85 (s, *Me*O-6) 3.95 (s, 2H, H1′ open chain **5a**), 6.29 (d, $J = 2.62$ Hz, H-5), 6.37 (d, $J = 2.0$ Hz, H-7), 11.80 (s, 1H, OH) ppm (for numbering see lactol form **5b**).

rac-4,6-Dihydroxy-2-(2-oxoheptadecyl) benzoic acid (**1a**) *or 3,6,8-Trihydroxy-3,4-dihydro-3-pentadecylisocoumarin (peniolactol)* (**1b**) ($C_{24}H_{38}O_5$)

To a stirred solution of 0.2 g **4ab** (46 mmol) in 6 cm^3 dry CH_2Cl_2 at -78°C was added dropwise 2.76 cm^3 1 *M* of a solution of BBr_3 (2.76 mmol) in dry CH_2Cl_2 under Ar. After stirring for 1 h at -78°C, the reaction mixture was warmed to room temperature and stirred for 24 h. Then it was poured into 50 cm^3 ice–H_2O. The layers were separated and the aqueous layer extracted with 2×50 cm^3 CH_2Cl_2 and then 50 cm^3 *Et*OAc. The combined organic phases were dried ($MgSO_4$) and concentrated. Flash chromatography (petrol ether:ethyl acetate = 6:4) afforded 0.102 g **1ab** as colourless prisms (55%) and some recovered isocoumarin **3**. Mp 147–149°C (Ref. [1] 150°C); MS: m/z (%) = 406 [M^+] (24), 388 (71), 195 (32), 178 (76), 167 (89), 150 (100); IR (film): $\nu = 3709$, 3598, 2922, 2853, 1710, 1653, 1459, 1260, 1081 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.87$ (t, $J = 7.2$ Hz, 3H, H-15′), 1.24 (brs, 24H, H3′–H14′), 1.57 (m, 2H, H2′), 2.35 (t, $J = 7.52$ Hz, 2H, H-1′), 2.86–2.65 (q, 2H, $J = 7.6$ Hz H-4 lactol form **1b**), 3.94 (s, 2H, H1′ keto acid form **1a**), 6.73 (s, H-5), 7.09 (s, H-7), 10.50 (br, 1H, 6-OH), 11.22 (br, OH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.2$ (C1), 110.2 (C3), 37.29 (C4), 144.5 (C4a), 107.0 (C5), 166.1 (C6), 99.5 (C7), 164.5 (C8), 101.3 (8a), 35.3 (C1′), 23.4 (C2′), 29.82, 29.8, 29.7, 29.67, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1 (C3′–C12′), 31.9 (C13′), 22.7 (C14′), 14.2 (C15′) ppm (C/H numbering according to lactol form **1b**).

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